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DOI:

[10.1016/j.envint.2016.08.021](https://doi.org/10.1016/j.envint.2016.08.021)

Document Version

Peer reviewed version

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Citation for published version (APA):

Walton, R. T., Mudway, I. S., Dundas, I., Marlin, N., Koh, L. C., Aitlhadj, L., Vulliamy, T., Jamaludin, J. B., Wood, H. E., Barratt, B. M., Beevers, S., Dajnak, D., Sheikh, A., Kelly, F. J., Griffiths, C. J., & Grigg, J. (2016). Air pollution, ethnicity and telomere length in east London schoolchildren: An observational study. *Environment International*, 96, 41-47. <https://doi.org/10.1016/j.envint.2016.08.021>

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**Air pollution, ethnicity and telomere length in east London schoolchildren:
an observational study**

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21 **Short running title: London air pollution and telomeres in children**

22 **Acknowledgements:** This study was funded/supported by the National Institute for Health
23 Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS
24 Foundation Trust and King's College London, Dr. and Mrs. Lee Iu Cheung Fund, and
25 Hackney Primary Care Trust (PCT). We thank Ms Michiru Mori for her assistance with
26 determination of salivary Ig A and cortisol.

27 **Competing financial interests declaration:** The authors declare they have no actual or
28 potential competing financial interests.

29 **Keywords**

30 Telomeres, air pollution, ethnicity, lung function, particulate matter, nitrogen oxides.

Abstract

Background: Short telomeres are associated with chronic disease and early mortality. Recent studies in adults suggest an association between telomere length and exposure to particulate matter, and that ethnicity may modify the relationship. However associations in children are unknown.

Objectives: We examined associations between air pollution and telomere length in an ethnically diverse group of children exposed to high levels of traffic derived pollutants, particularly diesel exhaust, and to environmental tobacco smoke.

Methods: Oral DNA from 333 children (8-9 years) participating in a study on air quality and respiratory health in 23 inner city London schools was analysed for relative telomere length using monochrome multiplex qPCR. Annual, weekly and daily exposures to nitrogen oxides and particulate matter were obtained from urban dispersion models (2008-10) and tobacco smoke by urinary cotinine. Ethnicity was assessed by self-report and continental ancestry by analysis of 28 random genomic markers. We used linear mixed effects models to examine associations with telomere length.

Results: Telomere length increased with increasing annual exposure to NO_x (model coefficient 0.003, [0.001, 0.005], p<0.001), NO₂ (0.009 [0.004, 0.015], p<0.001), PM_{2.5} (0.041, [0.020, 0.063], p<0.001) and PM₁₀ (0.096, [0.044, 0.149], p<0.001). There was no association with environmental tobacco smoke. Telomere length was increased in children reporting black ethnicity (22% [95% CI 10%, 36%], p<0.001)

Conclusions: Pollution exposure is associated with longer telomeres in children and genetic ancestry is an important determinant of telomere length. Further studies should investigate

both short and long-term associations between pollutant exposure and telomeres in childhood and assess underlying mechanisms.

Introduction

Short telomere length in circulating leucocytes is associated with common diseases that cause substantial mortality and morbidity across human populations (Calado and Young 2009). Environmental factors, particularly those inducing cellular oxidative stress, are thought to be important in determining the rate of telomere erosion (von Zglinicki et al. 2005). It has been suggested that exposure to air pollution causes oxidative stress (Miller 2014) and that vehicle emissions contribute significantly to the oxidative burden (De Prins et al. 2014; Rosa et al. 2014). Particulate matter collected from roadside locations in London, has remarkably high oxidative potential with significant contributions both from vehicle exhausts and mechanical abrasion of brakes and tyres (Kelly et al. 2011). Studies in adults have shown associations between short telomere length and traffic-related pollution: black carbon (McCracken et al. 2010; Pieters et al. 2015); aromatic hydrocarbons (Hoxha et al. 2009) although in one study the direction of the association was contradictory (Hou et al. 2012)

The long-term consequences of shortened telomeres on health are substantial (Grahame and Schlesinger 2012). There are strong associations with coronary heart disease (Brouillette et al. 2007; Codd et al. 2013) and studies in other cohorts show associations with all-cause mortality, which persist when estimates are adjusted for heart disease risk (Fitzpatrick et al. 2011), although these findings are not universal (Svensson et al. 2014). Meta analyses show that short telomeres in adults are associated with common solid tumours particularly bladder, oesophageal, gastric and renal (Wentzensen et al. 2011). Whilst shared environmental factors

and reverse causality might explain some of these associations, one recent large study in adults found a strong relationship between germline genetic determinants of telomere length and cancer risk which suggests a direct causal link (Iles et al. 2014).

The rate of telomere loss is greatest in young children (Aubert and Lansdorp 2008) and the decline in length then continues at a slower rate throughout adulthood (Yamaguchi et al. 2005). Thus telomere loss in childhood is a potentially important factor governing ultimate telomere length in adults. The effects of environmental factors might be expected to be greatest in childhood when most telomere attrition is occurring. However whilst there is some evidence that prenatal exposure to tobacco smoke has a lasting effect on telomere length (Theall et al. 2013), there are to date no studies examining associations between exposure to particulate matter and telomere length in children.

There is some preliminary evidence that telomere length in adults is related to continental ancestry, such that Africans have longer telomeres than Europeans (Needham et al. 2013). However ethnicity has not been considered in previous reports of environmental effects on telomeres arising from exposure to pollutants.

Based in an overview of the adult data, we hypothesised that telomere length in children would be inversely related to pollution exposure. Thus we examined associations between air pollution and telomere length in children from African, Asian and European ethnic backgrounds in an area of east London with high traffic density and a high proportion of diesel vehicles.

Methods

Study Design and Setting : Children aged 8-9 years in 23 schools in east London (Tower Hamlets and Hackney) participated in the EXHALE (Exploration of Health and Lungs in the Environment) study examining the impact of air pollution on respiratory health (Wood et al. 2015). Participating schools were selected to achieve a high contrast in urban pollutant exposure based on urban dispersion models at 20x20m resolution (London 2008). All children gave information on respiratory health using a standard questionnaire (ISAAC 1998) together with saliva and urine samples in a sequential cross-sectional study over three consecutive winters (Nov-Mar, 2008-11). Parents gave written consent and children verbal assent. The study was approved under research ethics and governance frameworks (Ref 08-H0704-139). The first year of the study was an internal pilot where study procedures for obtaining measurements and biological samples in schools were optimised.

Participants: All children on school registers with parental consent were eligible and were sent questionnaires. Assessments were conducted one day at each school, each year and those children not present were not followed up. Demographic data including ethnicity were obtained from school records. Deprivation score for each child was assigned by home postcode (<http://dclgapps.communities.gov.uk/imd/imd-by-postcode.html>). Height was measured using a portable stadiometer and recorded to 0.1 cm by trained investigators. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Level of obesity was classified using International Obesity Task Force criteria (Cole et al. 2000).

Air pollution: Annual exposures to NO_x, NO₂, PM₁₀ and PM_{2.5} were estimated using Kings College London, UK urban models (2008-2010) (Beevers et al. 2013), with residential and school address coordinates and assuming 15.6% time at school (7 hour school day, for 5 days per week, 39 weeks per year). Annual exposures were calculated as a calendar average for each year. Acute exposure estimates were derived at the address point by scaling annual mean concentrations according to a 'Nowcast' factor calculated for each pollutant for periods immediately prior to evaluation of lung function. The Nowcast factor is the ratio between concentrations measured by a local subset of continuous air pollution monitoring sites in the prior period, and the annual mean of measurements at the same sites. For this study 'Nowcast' scaling factors were calculated for the 24 hours and seven days before the school visits, working backwards from 10 am on the visit day to reflect both acute and sub-chronic exposure periods. To derive NO_x and NO₂, scaling factors measurements were averaged across 14-17 urban background and roadside sites within and surrounding the London Boroughs of Tower Hamlets and Hackney, based on data availability. For the PM₁₀ and PM_{2.5} scaling factors measurements from 9-13 and 14-20 background and roadside sites were averaged, respectively.

Environmental tobacco smoke: Urinary cotinine was measured by enzyme linked immunosorbant assay (ELISA) (Product number M155B1, Concateno, Abingdon UK) and corrected for creatinine (Product number 500701, Cayman Chemical Company, Ann Arbor, MI, USA). Children with a cotinine:creatinine ratio of ≥ 30 ng/mg were defined as positive for tobacco smoke exposure (Henderson et al. 1989).

IgA and cortisol: Salivary IgA was measured using a commercially available ELISA (eBioscience Easy Set-Go! ELISA 88-50600). Cortisol was determined by colorimetric competitive enzyme immunoassay (Enzo Life Sciences, ADI-901-071).

DNA and Genotyping: Genomic DNA was isolated from saliva (OrageneDNA kit OG-250, DNA Genotek Inc, Canada), quality assessed (Nanodrop ND-1000 Spectrophotometer, Nanodrop Technologies, Wilmington, DE), Quant-iT™ PicoGreen® assay (Invitrogen) and stored at -80 °C. DNA quality was confirmed by gel electrophoresis. Genotyping for randomly spaced markers was performed on multiple displacement amplified (MDA) DNA (REPLI g Midi Kit. Qiagen 150045) using GoldenGate genotyping assay on the IlluminaBeadXpress platform (Illumina Inc., San Diego, USA) and analyzed for assay quality control and Hardy Weinberg equilibrium with BeadStudio software. Genotyping success was 99%.

Continental ancestry: 27 randomly spaced single nucleotide polymorphisms were typed and population sub groups were assigned using the STRUCTURE algorithm (Pritchard et al. 2000). Markers were selected from the Hapmap data set using random numbers to locate chromosomal position. The closest marker to the position was selected unless this was known to be related to human disease in which case the next closest marker was chosen. Ten thousand iterations were performed with STRUCTURE for burn-in resulting in convergence with accurate allele frequency estimates. The process was repeated assuming between two and seven subpopulations with the best fit obtained assuming three population components. A numerical value representing each of these components was assigned to each child.

Telomere length: Telomere length was measured from oral DNA using a monochrome multiplex quantitative polymerase chain reaction (MMq-PCR) to compare telomere (T) repeat sequence copy number to a single copy gene (beta globin, S) (Cawthon 2009). Three reference DNA samples were included in each run as internal controls. Sample DNAs were assayed in triplicate and analysed against a standard curve, prepared using threefold serial dilutions of genomic DNA, also assayed in triplicate. MMq-PCR was performed using a

LightCycler480 as described previously (Vulliamy et al. 2011). Each reaction of 15µl contained 7.5µl SYBR Green I Master, 0.5µl of deionised water, 0.5µl for each the four primers (telg and telc at 30µM plus hbgu and hbgd at 6µM) and 5µl of DNA at 2ng/µl. A positive and negative control as well as a reference sample was included in each plate. Telomere length was expressed as T/S ratio based on the delta Ct (Ct telomere/Ct single-gene) derived from the standard curve and normalized to the reference sample.

Respiratory function: Spirometry was performed by trained investigators according to AT/S-ERS guidelines (Miller et al. 2005) with post-bronchodilator measurements of forced expiratory volume in 1 second (FEV1) reported after salbutamol 400 µg by large volume spacer. Flow-volume loops were manually inspected by an experienced reviewer (ID) for quality standards (Pellegrino et al. 2005).

Statistical methods: We hypothesised that telomere length would be inversely associated with exposure to outdoor air pollution and environmental tobacco smoke and that level of deprivation and obesity might modify this association.

All analysis was conducted according to a pre specified analysis plan. We used linear mixed models with a random effect for school to examine associations between relative telomere length and children's characteristics (Box1.). Characteristics included gender, reported ethnicity, body mass index, urinary cotinine and deprivation index adjusting for age, Ig A, cortisol and study year (**Model 1**). Variables found not to be associated (body mass index, urinary cotinine and deprivation score) were dropped from subsequent analysis. We investigated whether using genomic markers to determine continental ancestry instead of reported ethnicity was more informative (**Model 2**) and whether children's lung function was linked to relative telomere length (**Model 3**).

To assess associations between individual air pollutant exposures and relative telomere length we used linear mixed models with random effect for school crude (**Models 4-15**) and adjusting for age, sex, reported ethnicity, Ig A, cortisol and study year (**Models 16-27**). Measurements of telomere length were strongly positively skewed (Supplementary Figure 1) and therefore a log transformation was applied. The model coefficients in Tables 2 and 3 are ratios of geometric means which can be interpreted as percentage change. Associations between individual air pollutant exposures (Table 3) were presented per 1 unit ($\mu\text{g}/\text{m}^3$) increase in exposure and for the difference between the interquartile ranges (25th and 75th centile) of exposure.

200 DNA was successfully extracted from 988 of 1001 saliva samples collected during the first
201 three years of the EXHALE study, and of these 333 samples had sufficient genomic DNA for
202 telomere analysis (Figure 1). There were no telomere assay failures. Characteristics of the
203 children are reported in Table 1. There were no differences in baseline characteristics
204 between those who had sufficient DNA for telomere analysis and those who did not, apart
205 from a slight excess of boys (55% *v* 49%) (Supplementary Table 1). Median coefficient of
206 variation for the telomere and single copy gene determinations was 2.29 (range 0.08, 10.77)
207 and 0.84 (0.02, 4.39) respectively (Supplementary Figure 2). The median T/S ratio was 3.3
208 (range 1.7 to 9.1) and quartile coefficient of dispersion 45.5%.

Associations with telomere length: Model 1 in Table 2 shows that reported ethnicity is a major determinant of telomere length, with those reporting black ethnicity having higher T/S ratio than white or Asian children. The model coefficient represents a 22% (95%CI, 10%, 36%) increase in T/S ratio in children with black ethnic background compared to Asian. Girls had 8% lower T/S ratio than boys (95%CI, 2%, 14%). Body mass index, environmental tobacco exposure and index of multiple deprivation score were not associated with telomere length and were dropped from further models. **Model 2** includes information on continental ancestry from genomic markers and confirms that children with African ancestry have increased T/S ratio with a 10% increase in proportion of African ancestry resulting in a 1.6% increase in telomere length. **Model 3** shows an inverse relation between telomere length and respiratory function such that children with higher FEV₁ had a lower T/S ratio corresponding to 11% reduction (95%CI, 2% increase, 21% decrease) per litre of FEV₁. Ig A and Cortisol were not associated with relative telomere length but were nevertheless included in models to address possible confounding. There was no difference in exposure to pollution across ethnic groups (Supplementary Figure 3), in particular there was no association between increasing African ancestry and pollution exposure (Supplementary Figure 4).

Table 3 shows associations between telomere length and exposure to pollution. Children exposed to higher levels of nitrogen oxides and particulate matter had a higher T/S ratio than those experiencing lower levels. A 1µg/m³ increase in NO_x, NO₂, PM_{2.5} and PM₁₀ was associated with an increase in T/S ratio of 0.4%, 1.2%, 11.6% and 4.7% respectively. Comparing 25th to 75th centile for annual exposure to each pollutant: NO_x 2% increase in T/S ratio; NO₂ 4%; PM_{2.5} 12%; PM₁₀ 6%. The magnitudes of the associations were similar for exposures in the week before the assessment, but were absent when exposures in the previous day were considered.

Discussion

Main findings

In contrast to expectation long-term exposure to traffic related pollution is associated with increased telomere length in cells from salivary samples in children. The association is strongest with PM_{2.5} where children in the highest quartile of pollution exposure had a T/S ratio 15% higher than those in the lowest quartile. Whilst exposure to environmental tobacco smoke was highly prevalent in children taking part in this study (18%) this was not associated with telomere length.

In our highly ethnically diverse population, reported ethnicity was positively associated with telomere length, with black children having significantly longer telomeres than those of other ethnicities (22% black v Asian). We confirmed these findings using genomic markers related to continental origin to give a numerical representation of the proportion of ancestry from Africa, Asia and Europe.

Comparisons with other studies

This is the first study to examine a range of different air pollutants including nitrogen oxides and particulate matter (PM_{2.5}, PM₁₀) in the context of telomere length and the first to observe associations with telomere length in children. Previous studies have linked exposure to particulate matter with shorter telomeres in elderly men and suggested increased shortening with increasing age (McCracken et al. 2010). In contrast, one previous study in young adults showed an association between longer telomeres and short term exposure to particulate matter and suggested that longer exposures might be associated with shorter telomeres resulting from a balance between acute effects of inflammation and the longer term effects of oxidative stress (Hou 2012). Our results are consistent with these previous studies and

suggest that in children exposed continuously to high levels of pollution the lengthening effects may predominate.

In contrast to a recent large study in an ethnically diverse population of adults (Needham et al. 2013) we found no relation between low socio economic status and reduced telomere length. Many of the children in our study lived in areas of London suffering high levels of deprivation thus if there were an effect of deprivation on telomere length in children we would be likely to have observed it. Other studies in adults generally confirm a positive relationship between telomere length and socio economic status (Robertson et al. 2012; Surtees et al. 2012). One suggested mediator of this relationship is increased levels of stress (Mitchell et al. 2014) - adding salivary cortisol to the models as an approximation of current stress levels did not change the relation between pollution and telomere length in our study. Salivary Ig A was also included as a potential marker of depressed mucosal immunity, but as with cortisol it did not modify the underlying associations.

In children participating in our study telomere length was shorter in girls (8%) which is in contrast to previous studies in adults (De Meyer et al. 2007; Weischer et al. 2014). Since this is the first large-scale study on telomere length in children there are no direct comparisons. However the rate of telomere attrition is greater in men (De Meyer et al. 2007) and it may be therefore that boys have longer telomeres and then suffer a greater subsequent loss over the course of their lives. However the children in our study are unusual in being constantly exposed to high levels of air pollution, thus the observed telomere length may be related to this exposure and may not reflect telomere characteristics in more normal circumstances.

Potential mechanisms underlying the positive association between exposure to pollution and telomere length

DNA from saliva samples is derived mainly from peripheral blood leucocytes (Thiede et al. 2000). One mechanism to explain increased leukocyte telomere length in children exposed to pollution may be that an inflammatory response in the lungs leads to recruitment of circulating leucocytes and that oxidative stress, also related to pollution, results in apoptosis or cell death. Circulating leucocytes are then replaced with cells from the bone marrow at an earlier stage of differentiation, which because they have undergone fewer cell divisions, have longer telomeres. Previous studies have shown a direct relationship between proliferative potential of hematopoietic cells and telomere length, such that early progenitors have longer telomeres than terminally differentiated cells (Thiede et al. 2000). Longer telomeres during periods of severe oxidative stress have previously been observed in adults where telomere lengths subsequently normalised when the oxidative stress was removed (Shlush et al. 2011).

Another explanation could be that exposure to pollution induces telomerase which leads to increased telomere length. However granulocytes, which form the major proportion of circulating leucocytes, have very low telomerase activity (Weng 2001). It is possible that in childhood and early life the inflammatory effects of particulate matter are most important, whereas in later life the effects of oxidative stress on telomere attrition tend to dominate, as defences against oxidative stress attenuate. Whether this effect is compounded in adults by depletion of the lymphocyte pool and a limited capacity to replace cells damaged by oxidative stress is not known.

Strengths and weaknesses

Our study is to our knowledge the largest to date examining the effects of air pollution on telomere length, and the first to examine associations in children. The study is also the first to explore fully the effects of ethnicity on telomere length in children. The children in our study are likely to be representative of the global population with children from three major continents Europe, Africa and Asia. We also used a genomic measure of continental ancestry, which confirmed the effects of self-reported ethnicity on telomere length. This genomic measure also allows a degree of quantification of continental ancestry, which means that children reporting mixed ethnicity could be included in this analysis. Since ethnicity is a major determinant of telomere length, failure to account for genetic admixture in participants may have been a problem in previous studies (Mitchell et al. 2014).

We considered that environmental tobacco smoke, obesity and level of deprivation might modify the relationship between air pollution and telomere length however in our population these factors were unrelated to the outcome so were not included in the models. We adjusted models for IgA and cortisol to address potential confounding because of a strong *a priori* hypothesis that they would be related to telomere length.

Other sources of confounding which we are not able to account for are also possible, for example level of physical activity (Ornish et al. 2008). Height or body size could potentially confound the observed inverse relationship between telomere length and lung function since height is directly related to FEV1 and children with greater body size, who have necessarily experienced a greater number cell divisions, could potentially have shorter telomeres. There is some evidence to support this hypothesis from other species (Ringsby et al. 2015) although a paucity of existing data in children. We report crude FEV1 results rather than adjusted for height as this measure is generally preferred in children because of the greater variability in

the latter. The cross sectional design of our study in pre pubertal children minimises the effects of sexual dimorphism in body habitus related to higher oestrogen and testosterone levels.

Children were selected for telomere analysis on the basis of the volume of sample available. Whilst baseline characteristics of these children are comparable to those for whom sample volume was insufficient, we cannot be certain that telomere lengths were also similar.

Implications for future research

Future studies examining associations between air pollution and telomere length should account fully for effects of ethnicity. This may either be done by considering self-reported ethnicity or by using genomic methods to assign quantitative components of continental ancestry to each participant. The latter may be achieved either by using a panel of random genetic markers as described here or by using known ancestry informative markers. Interestingly, random markers perform well against sets of individually informative markers (Pardo-Seco et al. 2014) and offer the advantage that they make no presupposition about the continent of origin of study participants. There is also the potential problem that ancestry informative markers may be directly linked with disease or critical biological pathways for example the chemokine receptor CCR5 (Galvani and Slatkin 2003). Such associations may occur because of localised evolutionary pressure on different continents. Whilst such markers may be useful for forensic purposes, the link to disease and metabolic pathways may limit their application in biological studies. The random markers that we used were selected for their lack of known clinical associations.

Conclusion

346 Our studies are the largest conducted so far examining telomere length in children in relation
347 to exposure to air pollution. Since the baseline rate of loss is known to be greater in children
348 (Aubert and Lansdorp 2008), it is possible that children may be particularly susceptible to the
349 effects of environmental factors making this is an important area for further research. Early
350 exposure to pollution may thus have important effects on health in later life with
351 consequences for ageing and immunological senescence. Longitudinal studies would be
352 necessary to determine whether the children most affected by pollution exposure, who
353 experienced telomere lengthening in our study, will go on to have shorter leukocyte telomeres
354 in later life with attendant increased risk of chronic diseases.

References

- Aubert G, Lansdorp PM. 2008. Telomeres and aging. *Physiol Rev* 88:557-579.
- Beevers SD, Kitwiroon N, Williams ML, Kelly FJ, Ross Anderson H, Carslaw DC. 2013. Air pollution dispersion models for human exposure predictions in london. *J Expo Sci Environ Epidemiol* 23:647-653.
- Brouillette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, et al. 2007. Telomere length, risk of coronary heart disease, and statin treatment in the west of scotland primary prevention study: A nested case-control study. *Lancet* 369:107-114.
- Calado RT, Young NS. 2009. Telomere diseases. *N Engl J Med* 361:2353-2365.
- Cawthon RM. 2009. Telomere length measurement by a novel monochrome multiplex quantitative pcr method. *Nucleic Acids Res* 37:e21.
- Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. 2013. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet* 45:422-427, 427e421-422.
- Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. 2000. Establishing a standard definition for child overweight and obesity worldwide: International survey. *BMJ* 320:1240-1243.
- De Meyer T, Rietzschel ER, De Buyzere ML, De Bacquer D, Van Criekinge W, De Backer GG, et al. 2007. Paternal age at birth is an important determinant of offspring telomere length. *Hum Mol Genet* 16:3097-3102.
- De Prins S, Dons E, Van Poppel M, Int Panis L, Van de Mierop E, Nelen V, et al. 2014. Airway oxidative stress and inflammation markers in exhaled breath from children are linked with exposure to black carbon. *Environ Int* 73:440-446.

377 Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS, et al. 2011.
378 Leukocyte telomere length and mortality in the cardiovascular health study. *J Gerontol A*
379 *Biol Sci Med Sci* 66:421-429.

380 Galvani AP, Slatkin M. 2003. Evaluating plague and smallpox as historical selective
381 pressures for the ccr5-delta 32 hiv-resistance allele. *Proc Natl Acad Sci U S A* 100:15276-
382 15279.

383 Grahame TJ, Schlesinger RB. 2012. Oxidative stress-induced telomeric erosion as a
384 mechanism underlying airborne particulate matter-related cardiovascular disease. *Part Fibre*
385 *Toxicol* 9:21.

386 Henderson FW, Reid HF, Morris R, Wang OL, Hu PC, Helms RW, et al. 1989. Home air
387 nicotine levels and urinary cotinine excretion in preschool children. *Am Rev Respir Dis*
388 140:197-201.

389 Hoxha M, Dioni L, Bonzini M, Pesatori AC, Fustinoni S, Cavallo D, et al. 2009. Association
390 between leukocyte telomere shortening and exposure to traffic pollution: A cross-sectional
391 study on traffic officers and indoor office workers. *Environ Health* 8:41.

392 Iles MM, Bishop DT, Taylor JC, Hayward NK, Brossard M, Cust AE, et al. 2014. The effect
393 on melanoma risk of genes previously associated with telomere length. *J Natl Cancer Inst* 106.

394 ISAAC. 1998. Worldwide variation in prevalence of symptoms of asthma, allergic
395 rhinoconjunctivitis, and atopic eczema: Isaac. The international study of asthma and allergies
396 in childhood (isaac) steering committee. *Lancet* 351:1225-1232.

397 Kelly F, Anderson HR, Armstrong B, Atkinson R, Barratt B, Beevers S, et al. 2011. The
 398 impact of the congestion charging scheme on air quality in london. Part 2. Analysis of the
 399 oxidative potential of particulate matter. *Res Rep Health Eff Inst*:73-144.
 400 London Tf. 2008. London low emission zone impacts monitoring baseline report. London,
 401 UK.
 402 McCracken J, Baccarelli A, Hoxha M, Dioni L, Melly S, Coull B, et al. 2010. Annual
 403 ambient black carbon associated with shorter telomeres in elderly men: Veterans affairs
 404 normative aging study. *Environ Health Perspect* 118:1564-1570.
 405 Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. 2005.
 406 Standardisation of spirometry. *Eur Respir J* 26:319-338.
 407 Miller MR. 2014. The role of oxidative stress in the cardiovascular actions of particulate air
 408 pollution. *Biochem Soc Trans* 42:1006-1011.
 409 Mitchell C, Hobcraft J, McLanahan SS, Siegel SR, Berg A, Brooks-Gunn J, et al. 2014.
 410 Social disadvantage, genetic sensitivity, and children's telomere length. *Proc Natl Acad Sci U*
 411 *S A* 111:5944-5949.
 412 Needham BL, Adler N, Gregorich S, Rehkopf D, Lin J, Blackburn EH, et al. 2013.
 413 Socioeconomic status, health behavior, and leukocyte telomere length in the national health
 414 and nutrition examination survey, 1999-2002. *Soc Sci Med* 85:1-8.
 415 Ornish D, Lin J, Daubenmier J, Weidner G, Epel E, Kemp C, et al. 2008. Increased
 416 telomerase activity and comprehensive lifestyle changes: A pilot study. *Lancet Oncol* 9:1048-
 417 1057.

418 Pardo-Seco J, Martinon-Torres F, Salas A. 2014. Evaluating the accuracy of aim panels at
419 quantifying genome ancestry. *BMC Genomics* 15:543.

420 Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. 2005.
421 Interpretative strategies for lung function tests. *Eur Respir J* 26:948-968.

422 Pieters N, Janssen BG, Dewitte H, Cox B, Cuypers A, Lefebvre W, et al. 2015. Biomolecular
423 markers within the core axis of aging and particulate air pollution exposure in the elderly: A
424 cross-sectional study. *Environ Health Perspect*.

425 Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using
426 multilocus genotype data. *Genetics* 155:945-959.

427 Ringsby TH, Jensen H, Parn H, Kvalnes T, Boner W, Gillespie R, et al. 2015. On being the
428 right size: Increased body size is associated with reduced telomere length under natural
429 conditions. *Proc Biol Sci* 282:20152331.

430 Robertson T, Batty GD, Der G, Green MJ, McGlynn LM, McIntyre A, et al. 2012. Is
431 telomere length socially patterned? Evidence from the west of scotland twenty-07 study.
432 *PLoS One* 7:e41805.

433 Rosa MJ, Yan B, Chillrud SN, Acosta LM, Divjan A, Jacobson JS, et al. 2014. Domestic
434 airborne black carbon levels and 8-isoprostane in exhaled breath condensate among children
435 in new york city. *Environ Res* 135C:105-110.

436 Shlush LI, Skorecki KL, Itzkovitz S, Yehezkel S, Segev Y, Shachar H, et al. 2011. Telomere
437 elongation followed by telomere length reduction, in leukocytes from divers exposed to
438 intense oxidative stress--implications for tissue and organismal aging. *Mech Ageing Dev*
439 132:123-130.

440 Surtees PG, Wainwright NW, Pooley KA, Luben RN, Khaw KT, Easton DF, et al. 2012.
 441 Educational attainment and mean leukocyte telomere length in women in the european
 442 prospective investigation into cancer (epic)-norfolk population study. *Brain Behav Immun*
 443 26:414-418.

444 Svensson J, Karlsson MK, Ljunggren O, Tivesten A, Mellstrom D, Moverare-Skrtic S. 2014.
 445 Leukocyte telomere length is not associated with mortality in older men. *Exp Gerontol* 57:6-
 446 12.

447 Theall KP, McKasson S, Mabile E, Dunaway LF, Drury SS. 2013. Early hits and long-term
 448 consequences: Tracking the lasting impact of prenatal smoke exposure on telomere length in
 449 children. *Am J Public Health* 103 Suppl 1:S133-135.

450 Thiede C, Prange-Krex G, Freiberg-Richter J, Bornhauser M, Ehninger G. 2000. Buccal
 451 swabs but not mouthwash samples can be used to obtain pretransplant DNA fingerprints from
 452 recipients of allogeneic bone marrow transplants. *Bone Marrow Transplant* 25:575-577.

453 von Zglinicki T, Saretzki G, Ladhoff J, d'Adda di Fagagna F, Jackson SP. 2005. Human cell
 454 senescence as a DNA damage response. *Mech Ageing Dev* 126:111-117.

455 Vulliamy TJ, Kirwan MJ, Beswick R, Hossain U, Baqai C, Ratcliffe A, et al. 2011.
 456 Differences in disease severity but similar telomere lengths in genetic subgroups of patients
 457 with telomerase and shelterin mutations. *PLoS One* 6:e24383.

458 Weischer M, Bojesen SE, Nordestgaard BG. 2014. Telomere shortening unrelated to smoking,
 459 body weight, physical activity, and alcohol intake: 4,576 general population individuals with
 460 repeat measurements 10 years apart. *PLoS Genet* 10:e1004191.

461 Weng N. 2001. Interplay between telomere length and telomerase in human leukocyte
462 differentiation and aging. *J Leukoc Biol* 70:861-867.

463 Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA. 2011. The association of telomere
464 length and cancer: A meta-analysis. *Cancer Epidemiol Biomarkers Prev* 20:1238-1250.

465 Wood HE, Marlin N, Mudway IS, Bremner SA, Cross L, Dundas I, et al. 2015. Effects of air
466 pollution and the introduction of the london low emission zone on the prevalence of
467 respiratory and allergic symptoms in schoolchildren in east london: A sequential cross-
468 sectional study. *PLoS One* 10:e0109121.

469 Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. 2005.
470 Mutations in *tert*, the gene for telomerase reverse transcriptase, in aplastic anemia. *N Engl J*
471 *Med* 352:1413-1424.

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Box 1. Linear mixed models used to examine associations between telomere length and characteristics of the children

Abbreviations:

EtBl	Reported ethnicity: Black
EtWh	Reported ethnicity: White
EtMi	Reported ethnicity: Mixed/Other
BMI	Body mass index
ETS	Environmental tobacco
IMD	Deprivation score
PAs	Proportion of genetic ancestry: Asian
PAf	Proportion of genetic ancestry: African
AP	air pollutant exposure (each of NOx, NO2, PM2.5, PM10 measures annually, over previous week and over previous day)

i... indicator for child

j... indicator for school

u_j ... random effect, $u_j \sim N(0, \sigma^2_0)$

ϵ_{ij} ...error term, $\epsilon_{ij} \sim N(0, \sigma^2_e)$

σ^2_0 ...between school variance

σ^2_e ...within school variance

Model 1:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * sex_{ij} + \beta_2 * EtBl_{ij} + \beta_3 * EtWh_{ij} + \beta_4 * EtMi_{ij} + \beta_5 * BMI_{ij} + \beta_6 * ETS_{ij} + \beta_7 * IMD_{ij} + \beta_8 * age_{ij} + \beta_9 * IgA_{ij} + \beta_{10} * Cortisol_{ij} + \beta_{11} * Year2_{ij} + \beta_{12} * Year3_{ij} + u_j + \epsilon_{ij}$$

Model 2:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * sex_{ij} + \beta_2 * PAs_{ij} + \beta_3 * PAf_{ij} + \beta_4 * age_{ij} + \beta_5 * IgA_{ij} + \beta_6 * Cortisol_{ij} + \beta_7 * Year2_{ij} + \beta_8 * Year3_{ij} + u_j + \epsilon_{ij}$$

488 Model 3:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * sex_{ij} + \beta_2 * EtBl_{ij} + \beta_3 * EtWh_{ij} + \beta_4 * EtMi_{ij} + \beta_5 * PostFEV1_{ij} + \beta_6 * age_{ij} + \beta_7 * IgA_{ij} + \beta_8 * Cortisol_{ij} + \beta_9 * Year2_{ij} + \beta_{10} * Year3_{ij} + u_j + \epsilon_{ij}$$

490 Model 4 – 15

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * AP_{ij} + u_j + \epsilon_{ij}$$

492 Model 16-27:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * AP_{ij} + \beta_2 * sex_{ij} + \beta_3 * EtBl_{ij} + \beta_4 * EtWh_{ij} + \beta_5 * EtMi_{ij} + \beta_6 * age_{ij} + \beta_7 * IgA_{ij} + \beta_8 * Cortisol_{ij} + \beta_9 * Year2_{ij} + \beta_{10} * Year3_{ij} + u_j + \epsilon_{ij}$$

496 **Table 1. Characteristics of study population**

497

Variable	N obs	
Age (years), Mean(SD)	333	8.85 (0.33)
Female, N (%)	333	149 (44.7%)
Height (cm), Mean(SD)	333	133.67 (6.54)
Weight (kg), Mean(SD)	332	32.56 (8.11)
Body mass index (kg/m ²), Mean(SD)	332	18.04 (3.49)
International Obesity Task Force Grade, N (%)	332	
-2		4 (1.2%)
-1		21 (6.3%)
0		206 (61.9%)
1		62 (18.6%)
2		39 (11.7%)
Index of multiple deprivation score, Mean (SD)	332	44.6 (12.7)
Reported ethnicity, N (%)	333	
Asian		128 (38.4%)
Black		73 (21.9%)
White		88 (26.4%)
Mixed/Other		44 (13.2%)
Genetic ancestry components, Mean (SD)	327	
Asian		0.39 (0.29)
African		0.23 (0.35)
European		0.38 (0.28)
Environmental tobacco smoke exposure, N (%)	324	61 (18.8%)
Ig A (ng/ml), Mean(SD)	330	53700.94 (60149.12)
Cortisol, Mean(SD)	315	864.89 (1385.73)
Post bronchodilator FEV1 (L) d	327	1.68 (0.28)
Annual pollution exposures (µg/m ³)	333	
NO _x		76.12 (16.16)
NO ₂		43.59 (5.93)
PM _{2.5}		13.70 (0.82)
PM ₁₀		23.36 (1.53)

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500 **Table 2. Associations between telomere length, ethnicity and lung function**

Measure	Ratio of geometric means [95% CI]		
	Model 1 (N=305)	Model 2 (N=309)	Model 3 (N=309)
Female vs Male	0.917 [0.857,0.982]*	0.923 [0.863,0.987]**	0.914 [0.853,0.979]*
Reported ethnicity (Reference: Asian)			
Black	1.224 [1.104,1.358]**	-	1.222 [1.101,1.355]**
White	0.987 [0.892,1.093]	-	0.983 [0.893,1.083]
Mixed/Other	0.999 [0.889,1.123]	-	1.011 [0.900,1.137]
Body mass index	0.996 [0.986,1.006]	-	-
Environmental tobacco	1.000 [0.998,1.001]	-	-
Deprivation score	1.001 [0.998,1.004]	-	-
Continental ancestry (genomic markers)			
Asian	-	0.897 [0.769,1.046]	-
African	-	1.161 [1.014,1.330]*	-
Post bronchodilator FEV1 (L)	-	-	0.898 [0.792,1.019]

501 * p<0.05, ** p<0.001

502 The models were also adjusted for age, Ig A, cortisol and study year and included a random
503 intercept for school

504

Table 3. Associations between pollution exposure and telomere length (model coefficient [95% CI]).

Measure	Ratio of geometric means		
	Crude [95% CI]	Adjusted# [95% CI]	difference between 25 th and 75 th centile of exposure
	Models 4 – 15 (N=333)	Models 16 – 27 (N=315)	
Annual air pollution exposure (µg/m ³)			
NO _x	1.003 [1.001,1.005]*	1.004 [1.002,1.006]**	1.016
NO ₂	1.007 [1.001,1.013]*	1.012 [1.005,1.016]**	1.044
PM _{2.5}	1.007 [0.963,1.052]	1.116 [1.056,1.179]**	1.124
PM ₁₀	1.026 [1.004,1.049]*	1.047 [1.024,1.071]**	1.063
Exposure over previous week			
NO _x	1.004 [1.001,1.006]*	1.003 [1.000,1.006]*	1.052
NO ₂	1.008 [1.002,1.015]*	1.007 [1.000,1.014]	-
PM _{2.5}	1.016 [1.004,1.028]*	1.013 [1.000,1.025]*	1.051
PM ₁₀	1.013 [1.005,1.020]**	1.010 [1.002,1.018]*	1.053
Exposure over previous day			
NO _x	1.000 [0.999,1.002]	1.001 [0.999,1.002]	-
NO ₂	1.002 [0.997,1.006]	1.001 [0.997,1.006]	-
PM _{2.5}	1.003 [0.994,1.011]	1.004 [0.995,1.014]	-
PM ₁₀	1.002 [0.997,1.008]	1.003 [0.997,1.009]	-

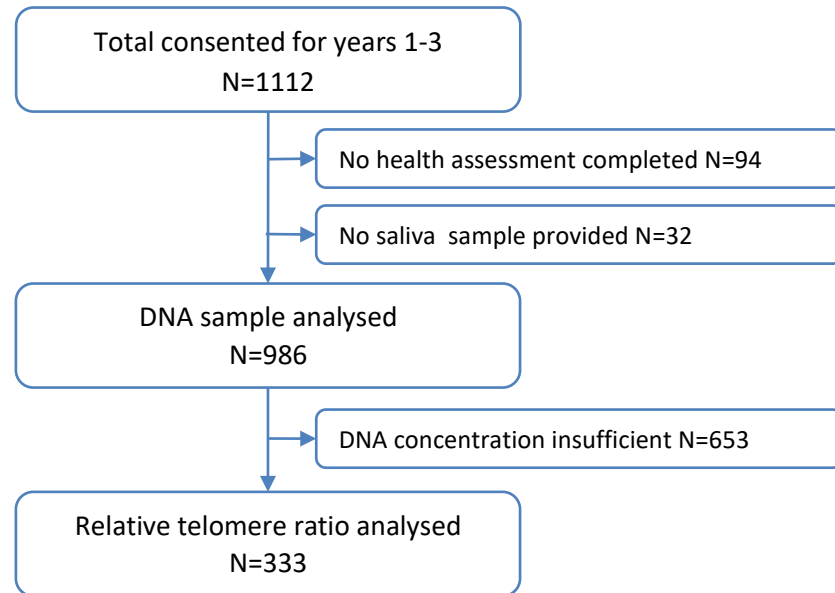
* p<0.05, ** p<0.001

The models were adjusted for age, sex, ethnicity, study year, IgA, cortisol and included a random intercept for school.

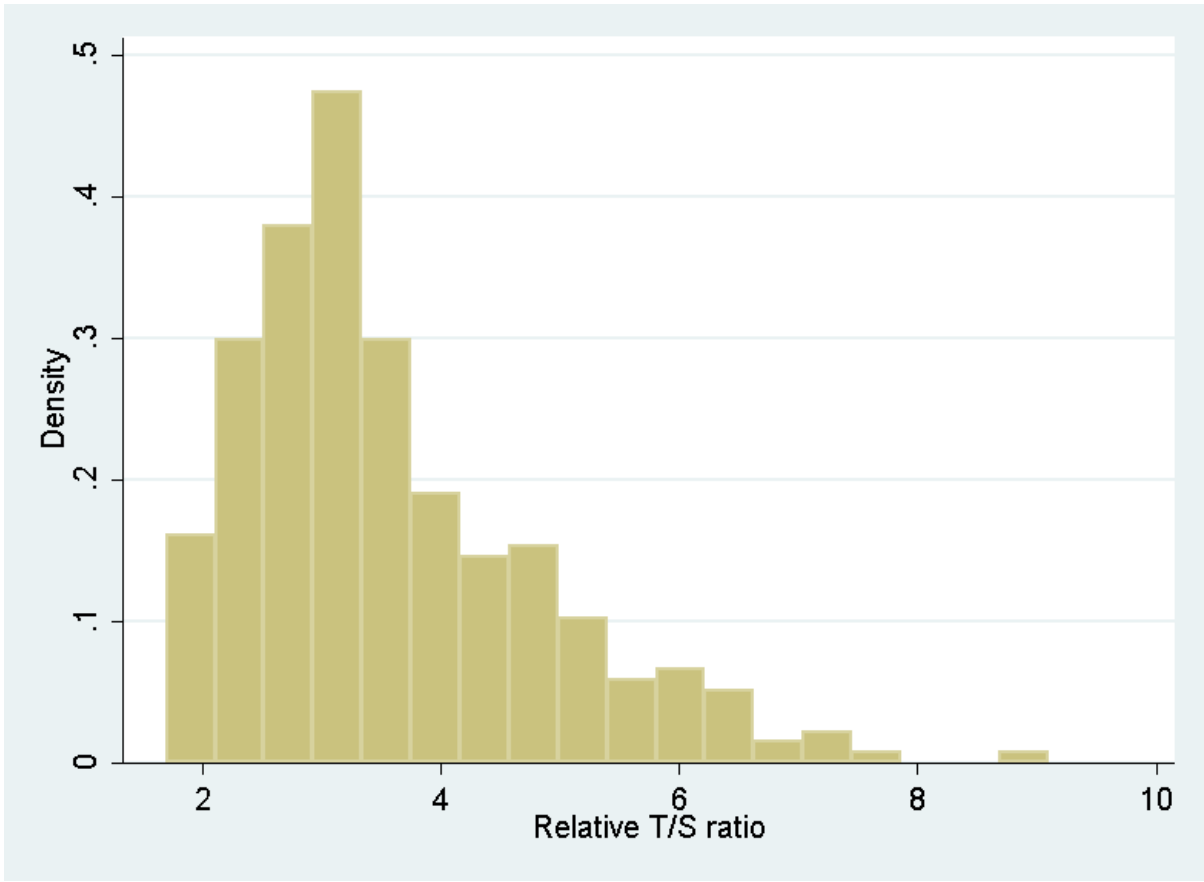
Supplementary Table 1

		Telomere data	
		not available	available
Age	Mean (SD)	N=779, 8.8 (0.3)	N=333, 8.9 (0.3)
Height	Mean (SD)	N=680, 133.8 (6.8)	N=333, 133.7 (6.5)
Weight	Mean (SD)	N=680, 32.5 (7.8)	N=332, 32.6 (8.1)
BMI	Mean (SD)	N=680, 18.0 (3.2)	N=332, 18.0 (3.5)
IMD score	Mean (SD)	N=776, 45.9 (10.4)	N=332, 44.6 (12.7)
Study year		N=779	N=333
	Year 1	202 (26%)	0 (0%)
	Year 2	334 (43%)	117 (35%)
	Year 3	243 (31%)	216 (65%)
Ethnicity		N=765	N=333
	Asian	275 (36%)	128 (38%)
	Black	197 (26%)	73 (22%)
	White	209 (27%)	88 (26%)
	Other	84 (11%)	44 (13%)
Gender		N=762	N=333
	Male	376 (49%)	184 (55%)
	Female	386 (51%)	149 (45%)
Asthma	N(%)	104/779 (13%)	39/333 (12%)
Environmental tobacco exposure	N(%)	157/646 (24%)	61/324 (19%)
Annual air pollution exposure (µg/m³)			
NO _x	Mean (SD)	N=775, 75.2 (13.4)	N=333, 76.1 (16.2)
NO ₂	Mean (SD)	N=775, 43.4 (5.1)	N=333, 43.6 (5.9)
PM _{2.5}	Mean (SD)	N=775, 13.7 (0.8)	N=333, 13.7 (0.8)
PM ₁₀	Mean (SD)	N=775, 23.4 (1.4)	N=333, 23.4 (1.5)

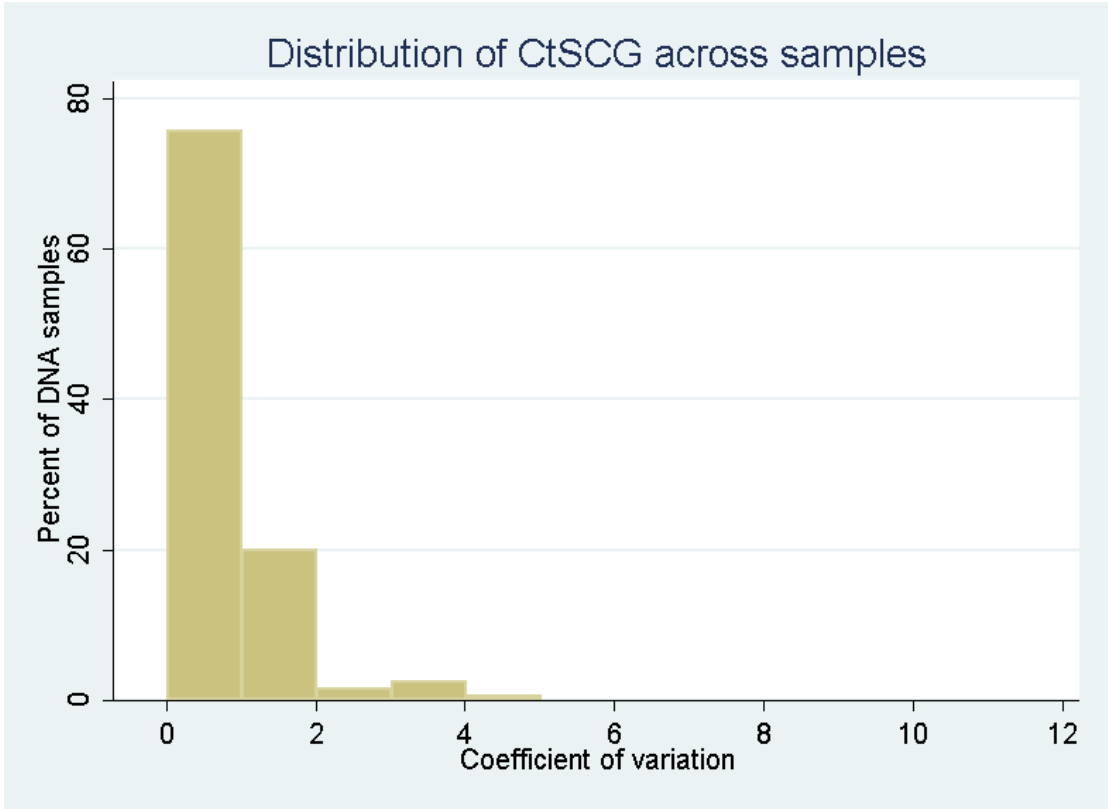
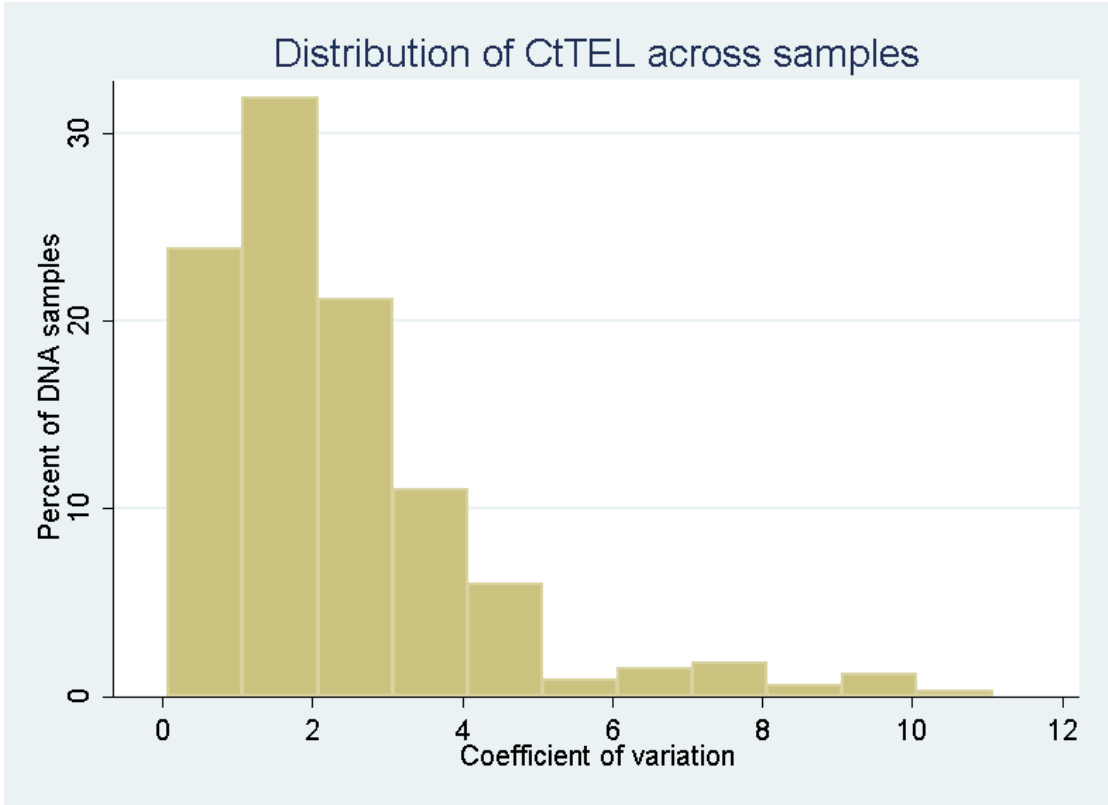
Figure 1. Flow of participants through the study



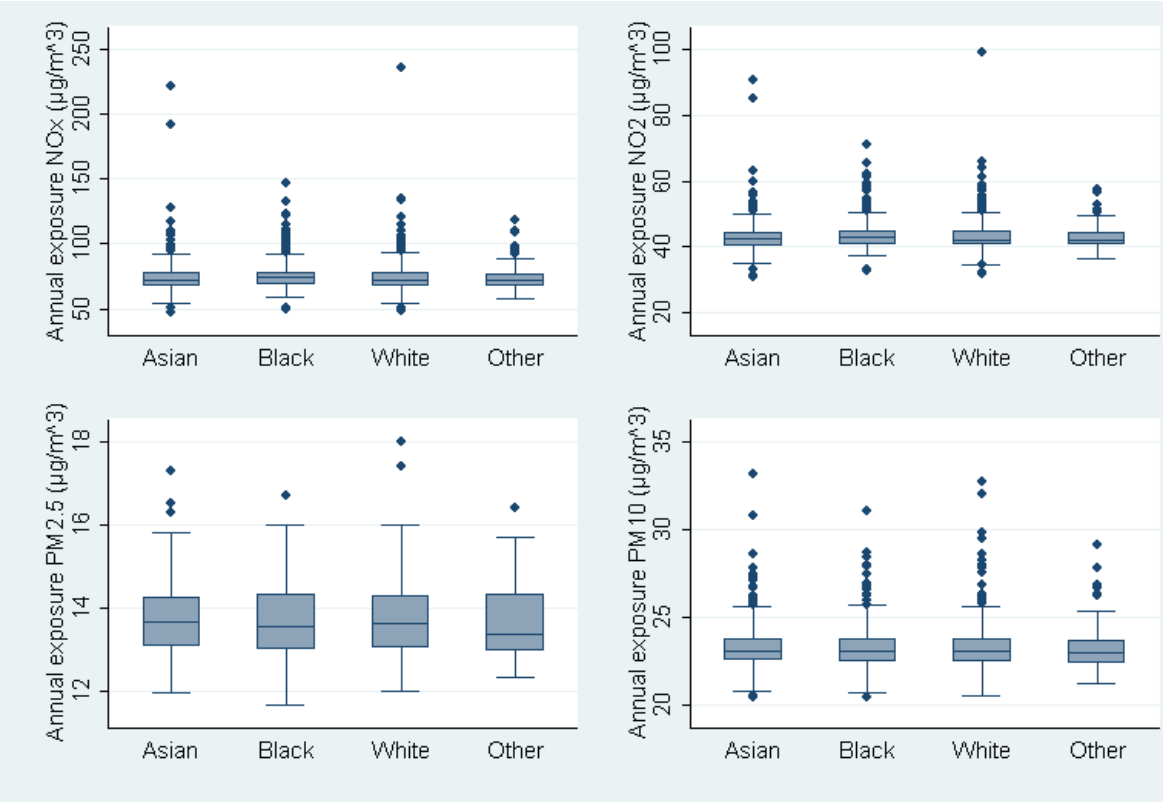
Supplementary Figure 1. Frequency distribution of relative telomere length (T/S ratio)



Supplementary Figure 2. Frequency distribution of the coefficient of variation for telomere and single copy gene determinations



Supplementary Figure 3. Annual pollution exposure by reported ethnicity



Supplementary Figure 4. Annual pollution exposure by proportion of African ancestry.

